/*----------START NOTEBOOK 2----------*/
Page: 1

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.l

/*----------*/
Page: 2

Date:. 7/9/14

Title:. Colony screen of merT

Purpose:. To colony screen for merT for shipping and to test the mini-prep sample

Methods:.
Mix the following reactions

Rxn 1: 2 rxns one with swab of each colony
0.5 ul F. Primer shipping vector seq F.
0.5 ul R. Primer shipping vector seq R
5 ul GoTaq
4 ul water
10 ul Total

Rxn 2: 2 rxns of this with swab of each colony
0.5 ul F. Primer shipping vector seq F.
0.5 ul R. primer merT (SpeI)
5 ul GoTaq
4 ul water
10 ul Total

Rxn 3: 2 rxns of this with swab of each colony
0.5 ul F. primer merT (XbaI)

0.5 ul R. primer merT (SpeI)
5 ul GoTaq
4 ul water
10 ul Total

Rxn 4:

0.5 ul F. primer merT (XbaI)
0.5 ul R. primer merT (SpeI)
5 ul GoTaq
0.5 ul mini-prep sample
3.5 ul water
10 ul Total

Perform 2 of each reaction 1-3 with 1 swab of colonies teal m4 and purple m2 in respective reaction.

Results:.

Conclusion:.

/*----------*/
Page: 3

Date:. 7/16/14

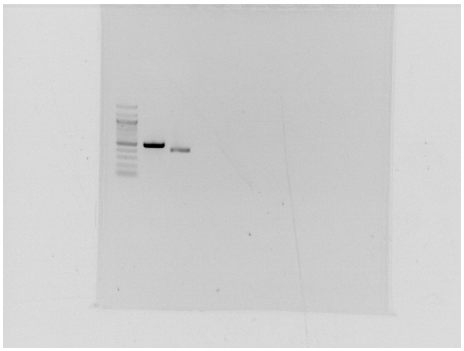
Title:. Amplification of merT with EcoRI and PstI

Purpose:. To amplify merT with different restriction enzymes to avoid worrying about stacking and orientation in shipping vector

Methods:.
Mix

1 ul pDU1358
1 ul merT F. primer 10nM (EcoRI)
1 ul merT R. primer 10nM (PstI)
25 ul Go Taq poly (2x)
0.5 ul pFU ultra DNA polymerase
22.5 ul water
51 ul total

Run PCR
Run gel @ 120v for 30 min

Results:. 

Conclusion:.

/*----------*/
Page: 4

Date:. 7/21/14

Title:.Rehydrate DNA for GFP study

Purpose:.To rehydrate DNA so our team cam complete the GFP study

Methods:.
Add 10 ul of NF water to

BBa_I20260 plate 4 well 18 A (km)
BBa_J23101plate 1 well 20 K (cm)
BBa_E0420 plate 2 well 24 B (cm)
BBa_J23115 plate 1 well 22 I (amp/cm?)

Results:.

Conclusion:.

/*----------*/
Page: 5

Date:. 7/22/14

Title:. Transformation of GFP study and ligation of merT

Purpose:. Transform *E. coli* UQ950 to complete the GRP study and ligate merT in order to transform it

Methods:.
Grab 2 100 ul samples of *E. coli* UQ950 and split into 4 aliquots
see notebook 1 page 29

Add

1.5 ul pSB1C3
1 ul merT
1 ul T4 Ligase Buffer
0.5 ul ligase
6 ul water
10 ul total

Incubate @ room temp f/ 1 hr

Results:.

Conclusion:. Cassandra transformed and plated *E. coli*.

/*----------*/
Page: 6

Date:. 7/24/14

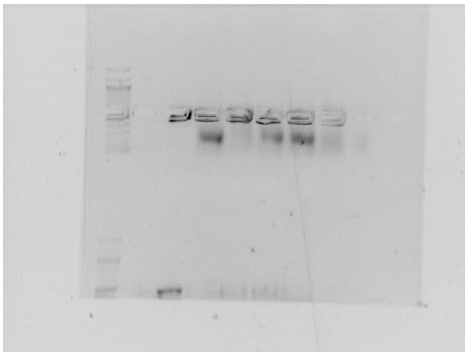
Title:. PCR colony screen merT

Purpose:. To find colonies that have merT

Methods:.
Mix

0.5 ul F. primer (EcoRI)
0.5 ul R. primer (PstI)
5 ul Go Taq
4 ul water
10 ul total x 6 (4 colonies, 1 +control, and 1 extra)

PCR w/ annealing temp @ 54^0C
Patch plate (cm)
Run gel



Results:.
0 colonies on less plate
5 colonies on more plate

Conclusion:.

/*----------*/
Page: 7

Date:. 7/28/14

Title:. Make 2 overnight cultures

Purpose:.To create more nanograms of DNA

Methods:.
Mix
- 5 ml LB
- 5 ul cm
- swab colony (2 & 3)

Let incubate in 37^0C

Results:.

Conclusion:.

/*----------*/
Page: 8

Date:. 7/26/14

Title:. Rattempt transformation for GFP study and mini-prep merT

Purpose:. To transform *E. coli* so they express certain genes from GFP study and to min prep yesterday overnight cultures.

Methods:.
Transformation
See notebook 1 page 29
Miniprep
follow directions from kit
except 50 ul of water instead of 75 ul TE buffer

Results:.
Transformation failed → no cells
colony 2 172 ng/ul
colony 3 36.7 ng/ul

Conclusion:.

/*--------------------------------*/
7-27-14
1. E. coli strains were streaked for singles on LB+Km plates for single colonies from -80 stocks. Strains tested: E. coli pBBRBB::mer, E. coli pBBRBB::gfp (vector control), E. coli pDU1358 (positive control).
/*--------------------------------*/

7-28-14
2. Single colonies were grown in 5 mL LB+Km (50ug/mL) overnight

/*--------------------------------*/

7-29-14
3. Overnight cultures were diluted 1/10 in minimal medium
4. 3 mL of diluted culture were spread evenly on Tryptone Medium plates. Three biological replicates were prepared for each strain.
Tryptone Medium per liter
15 g tryptone
5 g NaCl
10 g Noble Agar
1 pellet sodium hydroxide
1 L ddH2O

5. Plates were allowed to sit for 5 minutes after which excess culture was removed from each plate with a pipet

6. Plates were then allowed to dry for 10 minutes and filter disc was placed in the middle of each plate.

7. 10 uL of the stock 0.1M HgCl2 solution was then added to the filter disc. As a control, one plate was also prepared for each strain, and water was added to the filter to ensure nothing toxic was leaching from the filter disc.

8. Plates were incubated overnight at 37C

/*----------*/

7-30-14

9. Zones of inhibition were measured as the diameter of clearing around the filter disc. Two measurements were made for each plate, and three plates were tested for each strain.

/*----------*/

Page: 9

Date:. 8/4/14

Title:.Setting up sequencing reactions

Purpose:. to set-up reactions for sequencing

Methods:.
Rxn 1 and 2
2.91 ul DNA from colony 2
1.5 ul R. primer 6.4uM stock/F. primer merT 10 uM
7.59 ul water
12 ul total

Rxn 3 and 4
10.5 ul DNA from colony 3
1.5 ul R. primer 6.4uM stock/F. primer merT 10 uM
12 ul total

Results:. Successful sequencing was obtained

Conclusion:.

/*----------*/
Page: 10

Date:. 8/11/14

Title:. Amplification of pBBRBB with merA deletion

Purpose:. To prepare pBBRBB for Gibson assembly

Methods:.
Create 10 nM primers from new primers by 1:10 dilution

Mix
1 ul DNA
1 ul merT F. primer 10nM
1 ul merT R. primer 10nM
25.5 ul Go Taq poly (2x)
22.5 ul water
50 ul total x 2 rxn
PCR parts clon extension 6:00 min

Results:.

Conclusion:. Let sit @ 4^0C infinite then run gel to verify purity

/*----------*/
Page: 11

Date:. 8/11/14

Title:. Sequencing Rxn for merB

Purpose:.

Methods:.

	DNA (ul)	Primer	water	Colony #
LP1	7.1	1.5	3.4	2
LP2	7.1	1	3.9	2
LP3	7.1	1.5	3.4	2
LP4	7.1	1.5	3.4	3

LP5	7.1	1	3.9	3
LP6	6.7	1.5	3.8	3

LP1 and LP4: seq F
LP2 and LP5: merB seq1
LP3 and LP6: seq R

12 ul reactions

Results:.

Conclusion:.

/*----------*/
Page: 12

Date:. 8/12/14

Title:. Inoculation and gel verification

Purpose:.

Methods:.
Inoculate sample for David

Heat lid of LB and culture
dumped culture sans pipette tip into LB
placed in 37^0C shaker

Gel ran @ 120v for 45 min

Results:. Inconclusive; used wrong ladder

Conclusion:.

/*----------*/
Page: 13

Date:. 8/13/14

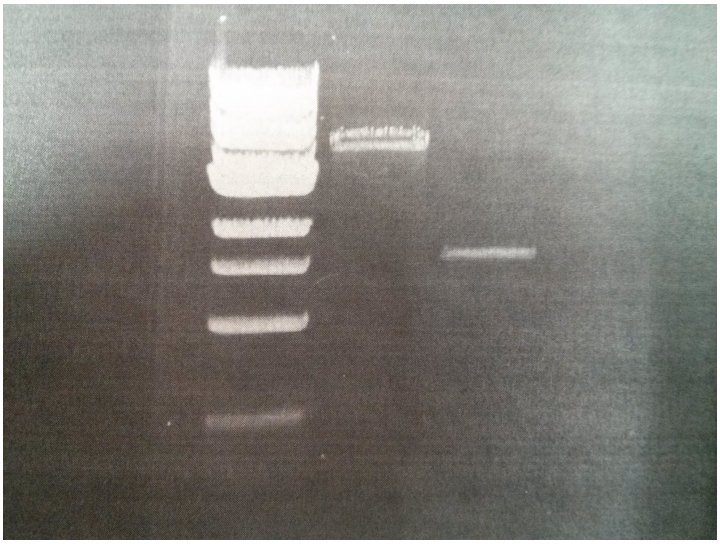
Title:. Reattempt gel verification

Purpose:. To correctly verify that the fragments are the correct length using the correct ladder

Methods:.
Gel 120V for 45 min

PCR purified according to the Invitrogen kit protocol.

Results:



Frag 1 62.1 ng/ul (4280 bp→ 0.022 pmol/ul)
Frag 2 40.4 ng/ul (1561 bp→ 0.039 pmol/ul)

Conclusion:.

/*----------*/
Page: 14

Date:. 8/15/14

Title:. Amplification of mer operon with biobrick restriction sites

Purpose:. To isolate mer operon with biobrick restriction sites in preparation for ligation into pSB1C3

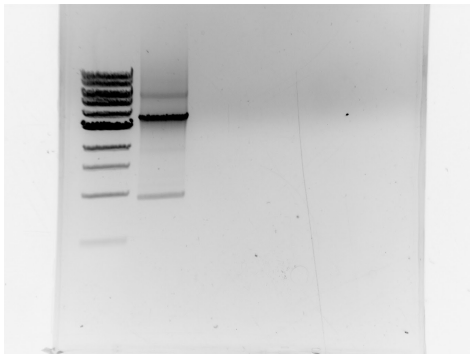
Methods:.
Prepare the following PCR reaction

1 ul pBBRBB::merRTPAB
1 ul merT F. primer 10nM
1 ul merT R. primer 10nM
25 ul Go Taq poly (2x)
0.5 ul Phusion DNA poly
22.5 ul water
50 ul total x 3 reactions

Thermal Cycler program

1	2	3	4	5	6	7
95^{0}C	95^{0}C	50^{0}C	72^{0}C	x28 2-4	72^{0}C	4^{0}C
2 min	30 sec	30 sec	4 min		5 min	oo

Results:. 3 ul 1 kb ladder, 5 ul mer amp product ran on 1 % agarose gel



Banding at ~3600 bp
consistent w size of mer operon
none-specific secondary products present

Conclusion:. Remaining 145 ul of pcr product must be used in gel extraction of mer insert.

/*----------*/
Page: 15

Date:. 8/18/14

Title:. Amplification of merA

Purpose:.

Methods:.

Mix

1 ul pBBRBB::mer colony 3
1 ul merA F. primer 10nM (EcoRI)
1 ul merA R. primer 10nM (PstI)
1.5 ul dNTPs
0.5 ul Phusion DNA polymerase
10 ul phusion buffer
36 ul water
50 ul total

Transform

see notebook 1 page 29

Results:.

DNA	Antibiotic	plate	# colonies
18 A	km	less	8
18 A	km	more	lawn
24 B	cm	less	75
24 B	cm	more	lawn
20 K	amp	more	-
20 K	cm	more	lawn
22 I	amp	more	-
22 I	cm	more	lawn

Conclusion:.

/*----------*/

Page: 16

Date:. 8/19/14

Title:. Continuation of GFP study

Purpose:.

Methods:.
Overnight cultures of 22I, 20K, and 18A
5 ml LB
5 ul antibiotic (depending on sample
swab colony
Let incubate overnight in 37^0C shaker

Add
300 ul LB
0.3 ul cm
swab of 24B
Let incubate overnight in 37^0C shaker

Mix
0.75 ul F. Primer
0.75 ul R. Primer
5 ul Go Taq
3.5 ul water
10 ul total
Run gel

Results:.

Conclusion:.

/*----------*/
Page: 17

Date:. 8/20/14

Title:. Ligation merA and merO and Miniprep GFP study

Purpose:.

Methods:.
Add
2 ul pSB1C3 (10.6 ng/ul)
6.5 ul merA
1 ul T4 Ligase Buffer
0.5 ul ligase
10 ul total

2 ul pSB1C3 (10.6 ng/ul)
2.67 ul merO
1 ul T4 Ligase Buffer
0.5 ul ligase
3.83 ul water
10 ul total

Incubate both @ room temp f/ 1 hr

Miniprep liquid cultures from yesterday (8/19) using invitrogen kit protocol
elute into 40 ul water

Run gel for PCR colony screen
Make 2 ligations

Results:.

DNA	Concentration (ng/ul)
merA	5.07
merO	10.6
18 A a	53.8
18 A b	42.1
20 K	315.0
22 I	40.6
24 B 1 less	4.64
24 B 2 less	9.6
24 B 3 more	5.56
24 B 4 more	6.31

Conclusion:.

/*----------*/
Page: 18

Date:. 8/21/14

Title:.Create an additional culture for GFP

Purpose:. Forgot to create patch plate, so need toredo a min-prep and create a patch plate5

Methods:.
Overnight Cultures (created 2)
Add
- 5 ml LB
- 5 ul cm
- swab of culture

Let incubate 37^0C shaker

Results:.

Conclusion:.

/*----------*/
Page: 19

Date:. 8/25/14

Title:.Recreate overnight cultures

Purpose:. Forgot to ask someone to remove overnight cultures so need to redo them.

Methods:.
Add
- 4 ml LB
- 4 ul cm
- swab of culture

Let incubate 37^0C shaker

Results:.

Conclusion:.

/*----------*/
Page: 20

Date:. 8/26/14

Title:.

Purpose:.

Methods:.
Miniprep according to Invitrogen kit
    eluted into 41 ul of water
Qubit
Digest
Mix
    2 ul Cutsmart
    0.5 ul EcoRI
    0.5 ul PstI
    5 ul DNA
    12 ul water
    20 ul total x 2
Incubate for 1 hour in 37^0C incubator

Run gel on all 20 ul @120V for 30min
Digest remaining miniprep w/ X/P
    2 ul Cutsmart
    0.5 ul XbaI
    0.5 ul PstI
    35 ul DNA
    9 ul water
    50 ul total for 24B

    2 ul Cutsmart
    0.5 ul SpeI
    0.5 ul PstI
    39 ul DNA
    5 ul water
    50 ul total for 22I and 20K
Incubate for overnight in 37^0C incubator
Reperform ligation

Results:.

Conclusion:.

/*----------*/

Date:. 8/27/14

Title:.

Purpose:.

Methods:.
Run gel for 24B @ 120v for 30 min
Extract smaller band @ ~876 bp

Column purify 22I and 20K
quantify everything

Overnight ligation
Transformation

Results:.
20 K 164 ng/ul
22 I 22.1 ng/ul

Conclusion:.

/*----------*/
Page: 22

Date:. 8/28/14

Title:.

Purpose:.

Methods:.
Ge purify 24B
Quantify 24 B
Ligate into 22I and 20K

PCR amplification
- 4 ul pDU1358
- 0.5 ul merT F. primer 10nM (EcoRI)
- 0.5 ul merT R. primer 10nM (PstI)
- 25.5 ul Go Taq poly (2x)

19.5 ul water
50 ul total

Results:.
24 B Less 13.8 ng/ul
24 B more 23.1 ng/ul

Conclusion:.

/----------/
Date: 9/20/14

Title:.

Purpose:.

Methods:.Methylmercury testing with encapsulated bacteria: Trial 1 – Purpose of experiment: determine if the *mer* operon in pBBRBB performs similar to pDU1358.

Added 7 mL of LB + Km (50mg/mL) to acid-washed glass Balch tubes

0.5 mL of encapsulated material containing the following strains were added to individual tubes:
Strains tested: *E. coli* K12 pBBRBB::*mer*, *P. putida* pBBRBB::*mer*
Negative Controls: *E. coli* K12 pBBRBB::*gfp*, *P. putida* pBBRBB::*gfp*
Positive Control*: P. putida* pDU1358

Cultures were incubated with shaking at 250 rpm and 37C and 30C for *E. coli* and *P. putida*, respectively.

Samples were taken after 36 hours of incubation and diluted one-million fold for concentration analysis. Methylmercury concentrations were measured with a Tekran model 2700 Automated Methyl Mercury Analyzer using EPA method 1630 without distillation. All quality assurance and quality control measures were taken as outlined in EPA method 1630. All MeHg standards (ongoing precision recoveries) were within the acceptable range averaging 96%.
/*----------*/
Date:. 10/1/14

Title:.

Purpose:.

Methods:.

Methylmercury testing with encapsulated and non-encapsulated cells: Trial 2 – purpose of experiment: determine rate of methylmercury degradation by encapsulated and non-encapsulated cells

Added 7 mL of LB + Km (50mg/mL) to acid-washed glass Balch tubes
Inoculation:

- For tubes containing encapsulated cells: 0.5 mL of encapsulated cell material were added to each tube
- For tubes containing non-encapsulated cells: overnight cultures were washed with minimal medium and brought to an OD = 1.0. Tubes were then inoculated to an initial OD ~0.08. Strains tested: *E. coli* K12 pBBRBB::*mer*, *E. coli* K12 pBBRBB::*gfp*, *P. putida* pBBRBB::*mer*, *P. putida* pBBRBB::*gfp*,
- Negative controls: Abiotic encapsulation material (to determine amount of methylmercury absorbed by beads), Abiotic medium (to determine the natural degradation rate for methylmercury), and *E. coli* K12 pBBRBB::*gfp, P. putida* pBBRBB::*gfp*
-

Methylmercury chloride(0.1M) was added to each tube at 1 mg/L. Non-encapsulated cells were also tested at 1 mg/mL and 4 mg/mL starting concentrations to determine degradation rate at differing starting concentrations.

Cultures were incubated at 250 rpm and 37C or 30C for *P. putida* and *E. coli* K12, respectively.

Samples were removed after 1, 2, 4, 8, and 24 hours. Methylmercury concentrations were measured with a Tekran model 2700 Automated Methyl Mercury Analyzer using EPA method 1630 without distillation. All quality assurance and quality control measures were taken as outlined in EPA method 1630. All MeHg standards (ongoing precision recoveries) were within the acceptable range averaging 96%.

/*----------*/
Date: 10-5-14

Methods:

1. Strains were streaked for singles on LB+Km plates for single colonies from -80 stocks. Strains tested: E. coli pBBRBB::mer, E. coli pBBRBB::gfp (vector control), E. coli pBBRBB::merΔmerA (to test for merPT transport and extra sensitivity of merA deletion strains to HgII), P. putida pBBRBB::mer, P. putida pBBRBB::gfp (vector control)

/*----------*/

10-6-14
2. Single colonies were grown in 5 mL LB+Km (50ug/mL) overnight

/*----------*/

10-7-14
3. Overnight cultures were diluted 1/10 in minimal medium
4. 3 mL of diluted culture were spread evenly on Tryptone Medium plates. Three biological replicates were prepared for each strain.

Tryptone Medium per liter
15 g tryptone
5 g NaCl
10 g Noble Agar
1 pellet sodium hydroxide
1 L ddH2O

5. Plates were allowed to sit for 5 minutes after which excess culture was removed from each plate with a pipet

6. Plates were then allowed to dry for 10 minutes and filter disc was placed in the middle of each plate.

7. 10 uL of the stock 0.1M HgCl2 solution was then added to the filter disc. As a control, one plate was also prepared for each strain, and water was added to the filter to ensure nothing toxic was leaching from the filter disc.

8. Plates were incubated overnight at 37C (E. coli) and 30C (P. putida).

/*----------*/

10-8-14

9. Zones of inhibition were measured as the diameter of clearing around the filter disc. Two measurements were made for each plate, and three plates were tested for each strain.